Neuroendocrine Control of Ecdysis in Silkmoths

James W. Truman & Lynn M. Riddiford

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It's 1970. Anecdotally, entomologists and collectors already know that butterflies and moths emerge from their cocoons at certain times of day - timing that varies between the species – but it was unclear what drives this process. By performing the first clock transplants, Truman & Riddiford produced a landmark study in the field of chronobiology to answer this question. So what exactly did they do?

Using different species of silk moth, Truman & Riddiford first characterised the emergence patterns of pupae turning into moths (ecdysis, or eclosion). They did this by asking two initial questions: 1) What time of day do these moths emerge? 2) What is the behaviour that accompanies this emergence? In answering these questions, they first saw that each species had a distinct window of time in which they were most likely to emerge (*H. cecropia* in the morning, *A. pernyi* in the evening) confirming reports of species-specific eclosion patterns. Each species also exhibited a distinct pattern of behaviour that preceded emergence, recorded using a kymograph. To determine the underlying mechanism driving these eclosion rhythms, they then focussed on one species (*H. cecropia*) and performed a variety of surgical methods in the silk moth pupae. Whilst severing the optic nerve or various ganglia had no effect, removal of the brain rendered the moths arrhythmic.

'What, then, is the role of the brain?'

The next series of experiments were a significant advance in understanding how the clock integrates with input and output pathways to generate rhythmic physiology. Truman & Riddiford removed the brains from pupae and transplanted them into the abdominal cavity to generate 'loose-brain' pupae. These 'loose-brain' moths retained the pattern of eclosion – the brain did not have to be connected to exert its effects! Developing a novel housing system, they then exposed the pupae to different lighting regimes in different areas of the body – the head in light when the body was in darkness and vice versa (Fig. 1). Whether the brain was in the head or the abdomen, the timing of eclosion matched the lighting schedule of the brain – the brain itself was sensing the photoperiod and driving eclosion to match its input.

Taking this a step further, they used the knowledge that each species had their own specific eclosion window (or phase) and tested what would happen if they mixed brains from one species with the body of another (Fig. 2). This inter-species transplant revealed multiple pieces of information. Firstly, it confirmed that the moth eclosion timing was determined by the brain – *H. cecropia* with *A. pernyi* brains emerged at the same time as intact *A. pernyi*. Secondly, it showed that the behavioural pattern which accompanied eclosion was determined by the rest of the body – transplanting the brain affected the timing, but not the stereotyped emergence behaviour. Third, it revealed that the brain output that triggered eclosion in these moths was not species- or even genus-specific. Given that the implanted brains could not re-establish neural connections, it was determined that a hormonal signal is the agent behind this process. This signal could be interpreted by the other moth, implying a common output whose release is timed by the brain but interpreted by the body to drive a distinct rhythmic pattern.

'The components include a photoreceptor mechanism, a clock, and a neuroendocrine output.'

The concepts established in this study were a landmark for rhythmic neuroendocrinology. Hormone secretion is rhythmic in many species, with cortisol, adrenaline and melatonin being perhaps the most well-known in human physiology. The idea that these secretions are timed by the brain, which is detecting a light-dark schedule and synchronising its activity to this, is central to this paper and holds true across multiple kingdoms. Although higher organisms would not be able to function in a 'loose-brain' scenario, the notion that a rhythmic output pattern could be generated by the brain clock which would continue to display this rhythm in a new setting has since been confirmed in other species.

It would be another 12 years before Terry Page transplants the clock in cockroaches[1], and then 8 more before Martin Ralph & Michael Menaker transplant the suprachiasmatic nuclei in hamsters[2]. Now that technology has advanced to the point of cell-specific genetic manipulations of the clock[3], it is rare to see transplantation studies such as these. In fact, researchers have even been able to introduce molecular oscillators to non-circadian species[4]. However, these experiments paved the way for our understanding of circadian hierarchies, input/output pathways and peripheral interpretation of rhythmic signals.

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[1] Page TL (1982) Transplantation of the Cockroach Circadian Pacemaker. *Science* 216:73-75. DOI: 10.1126/science.216.4541.73

[2] Ralph MR, Foster RG, Davis FC, Menaker M (1990) Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247:975-978. DOI: 10.1126/science.2305266

[3] Storch KF, Paz C, Signorovitch J, Raviola E, Pawlyk B, Li T, Weitz CJ (2007) Intrinsic circadian clock of the mammalian retina: importance for retinal processing of visual information. *Cell* 130(4):730-741. doi: 10.1016/j.cell.2007.06.045

[4] Chen A, Lubkowicz D, Yeong V, Chang L, Silver P. (2015) Transplantability of a circadian clock to a noncircadian organism. *Science Advances* 1(5):e1500358. DOI: 10.1126/sciadv.1500358

Figures

NB – the right to reproduce the real figure on a website is 30 euros (which I am happy to pay, but please do not post the figure image before confirmation). I can also just adapt figure 2 to not show any of the original data if desired.

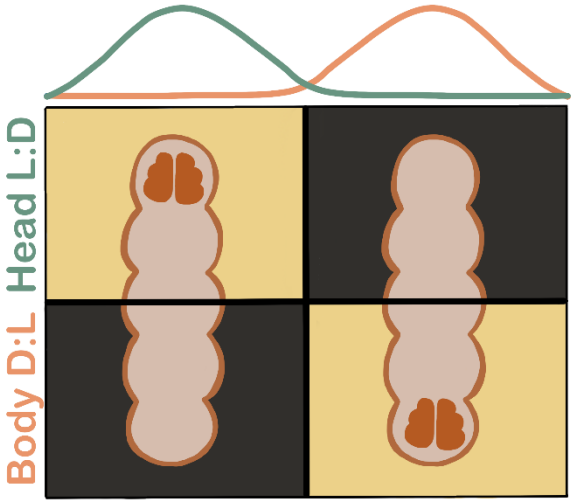

Figure 1: The silk moth brain senses light and determines the timing of eclosion.
Silk moth pupae were housed in special boxes where the head and body could be exposed to different light schedules – when the head is in the light, the body is in the dark and vice versa. By transplanting the brain to the abdomen, it was shown that the brain itself senses the light pattern and drives the timing of eclosion to match.

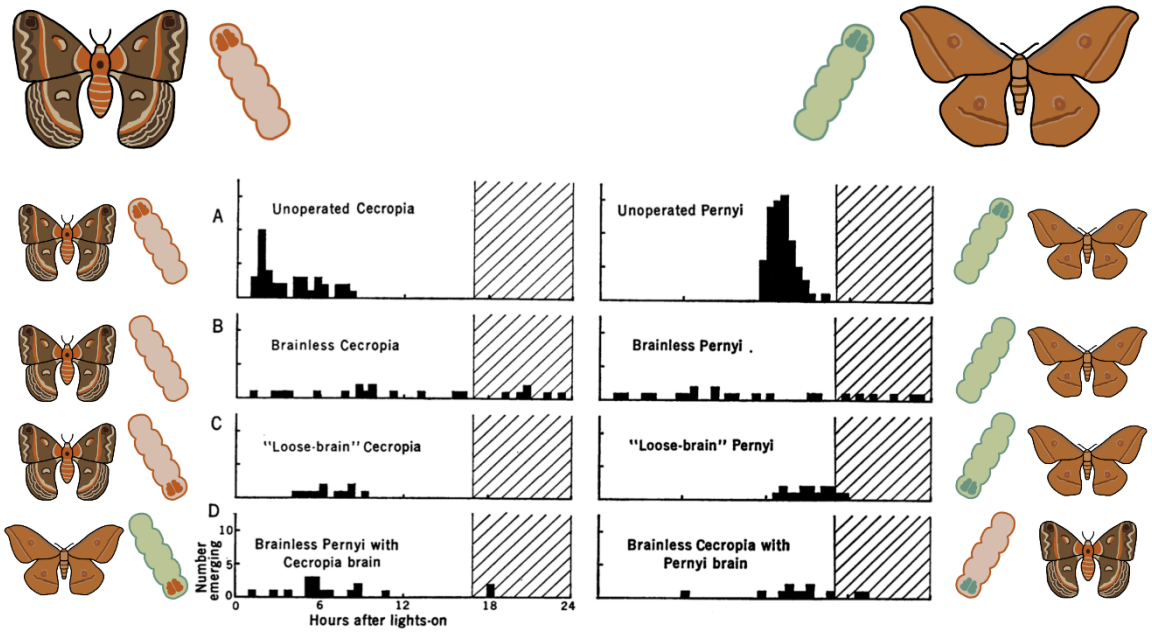

Figure 2: Inter-species transplants show that the brain controls the timing of silk moth eclosion.

A) Rhythm of eclosion in two silk moth species. B) Loss of eclosion rhythm in brainless silk moths. C) Timing is restored if a 'loose-brain' (brain transplanted into the abdomen) is present. D) A brain transplanted into a different silk moth species imparts its rhythm on the recipient.

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